A Waterborne Outbreak of Norwalk-Like Virus among Snowmobilers—Wyoming, 2001

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In February 2001, episodes of acute gastroenteritis were reported to the Wyoming Department of Health from persons who had recently vacationed at a snowmobile lodge in Wyoming. A retrospective cohort study found a significant association between water consumption and illness, and testing identified Norwalk-like virus (NLV) in 8 of 13 stool samples and 1 well. Nucleotide sequences from the positive well-water specimen and 6 of the positive stool samples were identical. This multistrain NLV outbreak investigation illustrates the importance of NLV as a cause of waterborne illness and should encourage monitoring for NLVs in drinking water.

Norwalk-like viruses (NLVs) are the major cause of viral gastroenteritis among adults worldwide [1]. Person-to-person transmission of NLVs is common, but these viruses also have caused extensive food- and waterborne outbreaks [2–4]. Waterborne outbreaks of NLV have been associated with a variety of sources, including municipal and private water systems [3, 4]. Data published elsewhere have shown that 23% of waterborne outbreaks of acute gastroenteritis reported to the Centers for Disease Control and Prevention (CDC) were clinically and epidemiologically consistent with disease caused by NLV [5].

It has been suggested that NLV is resistant to chlorine [6]. Because groundwater sources supply drinking water for ~130 million Americans and because NLVs may be infective even in chlorinated drinking water, these viruses have obvious importance in outbreaks of waterborne disease [7]. Methods for detection and isolation of enteric viruses in water samples have improved dramatically in recent years, creating greater opportunities to study the environmental transmission and true significance of NLVs [8–10]. The present report describes the investigation of a multistate NLV outbreak traced to a sewage-contaminated well.

Methods. In early February 2001, the Wyoming Department of Health began to receive reports of acute gastroenteritis from persons who had recently visited a snowmobile vacation lodge (lodge A) in Wyoming. Guests at lodge A often also visit 2 neighboring lodges (lodges B and C). Illness was characterized by nausea, vomiting, and diarrhea that were of brief duration. An investigation was conducted to determine the etiologic agent, examine exposures, and identify the mode of transmission.

A retrospective cohort study was conducted among guests at lodges A and B; lodge C declined to participate in the epidemiological investigation. A list was obtained of all lodge guests with contact information who visited lodge A from 31 December 2000 through 9 March 2001 and who visited lodge B from 15 January through 15 February 2001. A smaller sample was chosen for lodge B guests, from what was perceived as the height of the outbreak, as a comparison cohort analysis and to rule out the possibility that lodge B was the source of the outbreak.

Lodge guests were interviewed by telephone, using a standardized questionnaire. Interview questions covered symptoms, dates of illness, water and ice consumption, and a complete list of menu items served at the lodge A restaurant. A case of acute gastroenteritis was defined as development of vomiting or diarrhea (≥3 loose stools within a 24-h period) in a guest of either lodge. Data were analyzed using Epi Info version 6.04d software (CDC) [11].

Bulk stool samples from 13 lodge guests who resided in 3 states were tested for NLV by reverse-transcriptase polymerase chain reaction (RT-PCR) using a region B degenerate primer

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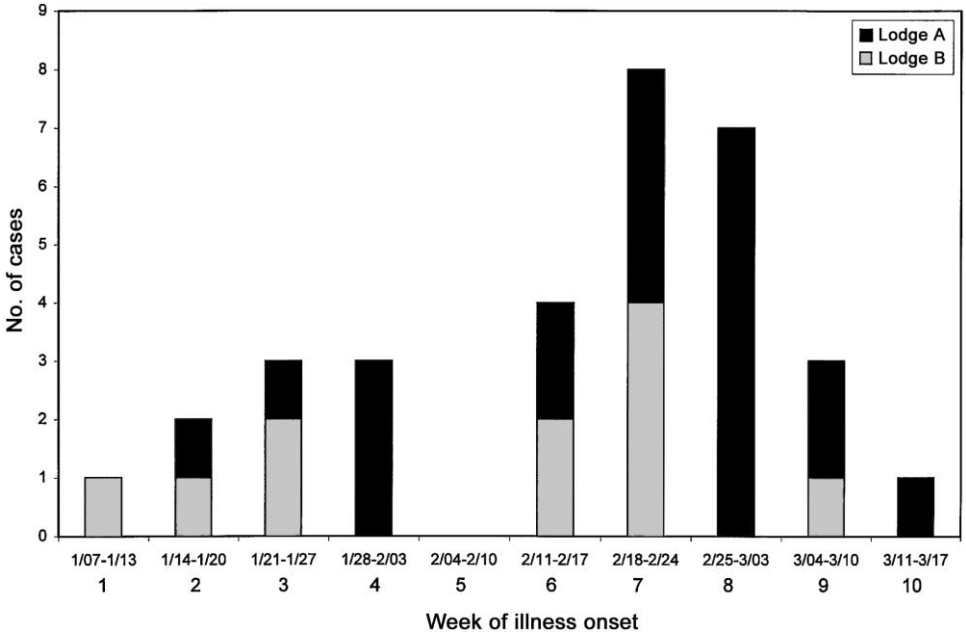

Figure 1. Dates (month/day) of onset of gastroenteritis among snowmobilers at 2 lodges in Wyoming in 2001

set to amplify a 213-bp portion of the NLV RNA polymerase gene (R. Fankhauser, personal communication). In brief, reactions were performed with a set of degenerate primers that included NLV431 (tggacIagRggIccYaaYca), NLV432 (tggacIcgYggIccYaaYca), NLV433 (gaaYctcatccaYctgaacat), and NLV434 (ggaScgcatccaRcggaacat). Positive samples were further characterized by nucleotide sequencing. Stool specimens also were tested for the presence of *Salmonella, Shigella, Campylobacter,* and *Escherichia coli* O157:H7.

Samples of well water from lodge A were collected from 1 of 3 on-site wells and tested for the presence of NLVs and fecal coliforms. A 946-L water sample was concentrated using a 1MDS cartridge filter (Cuno) [12] and processed to detect virus. In brief, viruses were eluted from the filters and concentrated using a celite method [13]. The celite concentrate was treated to remove inhibitors of PCR (G. S. Fout, B. C. Martinson, M. W. N. Moyer, D. R. Dahling, and L. J. Wymer, unpublished data). Caliciviruses present in the treated concentrate were amplified using the region B degenerate primer set, and NLV-positive products were cloned and sequenced. Well-water specimens from lodges B and C also were tested for the presence of fecal coliforms.

Food handlers working in the lodge A restaurant were questioned about procedures used in the preparation of food items and history of illness. An assessment of the lodge's water supply system and sewage disposal system was conducted.

Results. One hundred twelve persons were identified as having registered a party of $\geqslant$1 persons at lodge A between 31 December 2000 and 9 March 2001. Fifty-four guests of lodge A and 27 guests of lodge B were interviewed; these study participants were selected to participate either because contact information for them was available from lodge records or because their names and phone numbers were given as party members by an interviewed person.

Forty-one percent of respondents (22 of 54) from lodge A and 48% of respondents (13 of 27) from lodge B developed acute gastroenteritis during or within 1 week of their stays. Of the lodge guests surveyed in the cohort study, the first case patient developed symptoms on 13 January 2001, and cases continued to be reported until 9 March, when the lodge was closed by the Wyoming Department of Health pending sanitary improvements (figure 1).

The symptoms most commonly reported by case patients were diarrhea (86%), nausea (86%), abdominal cramps (80%), and vomiting (70%). The duration of illness ranged from 1 to 9 days (median, 2 days); 88% of the case patients were ill for $\leqslant$5 days. Seven guests sought medical care, and 1 person was hospitalized. Ill persons ranged in age from 15 to 84 years (median, 37 years).

Among lodge A guests, illness was significantly associated with water consumption (relative risk [RR], 3.3; 95% confidence interval [CI], 1.4–7.7). A χ^2 test for linear trend was performed, and the results indicated that the risk of illness increased with the number of glasses of water consumed ($P = .0003$; table 1). No food items were associated with illness.

Among lodge B guests, water consumption at lodge B was not associated with illness. However, guests of lodge B who ate or drank at lodge A had a significantly greater risk of illness than guests who did not (12 of 16 guests who visited lodge A and 1 of 10 guests who did not visit lodge A were ill [RR, 7.5; 95% CI, 1.1–49.2]).

Table 1. Risk of acute gastroenteritis associated with quantity of water consumed among snowmobilers who vacationed at 2 lodges in Wyoming in 2001.

No. of glasses of water consumed	No. of ill persons/ total no. (%)[a]	RR (95% CI)
0	6/29 (21)	1.0 (reference)
1–3	6/14 (43)	2.07 (0.8–5.3)
4–5	5/6 (83)	4.03 (1.8–9.0)
>5	3/3 (100)	—[b]

NOTE. CI, confidence interval; RR, relative risk.

[a] χ^2 test for linear trend, 13.3; $P = .0003$.

[b] Undefined because the denominator is 0.

Of the 13 stool specimens collected from lodge guests who were ill, 8 (62%) tested positive for NLV by RT-PCR (4 of 4 stool specimens from case patients who lived in North Dakota, 2 of 2 from case patients who lived in Minnesota, and 2 of 7 from case patients who lived in Wyoming). The positive specimens from North Dakota and Minnesota all contained strains with an identical sequence and belonged to NLV genogroup II. The PCR products from viral RNA in the 2 positive specimens from Wyoming also belonged to NLV genogroup II, but each had a unique sequence. A total of 3 distinct sequence types were detected in the outbreak. No bacterial pathogens were identified in the stool samples that were examined.

Seven of 8 well-water samples obtained from lodge A on 13 March 2001 tested positive for fecal coliforms. Testing of well water at lodges B and C showed no evidence of bacterial contamination. NLV was detected, using RT-PCR amplification techniques, in the 1MDS concentrate from a well-water sample collected on 19 March at lodge A. Three separate clones were sequenced, and all were identical in the 173-bp region between the primers, except for a single base insertion in 1 clone at nucleotide position 40. The consensus sequence without the insertion was identical to the corresponding portion of NLV genome that was amplified from the stool specimens collected from the North Dakota case patients on 20 and 21 February and from the Minnesota case patients on 14 and 16 March.

Lodge A was served by 3 on-site groundwater wells that reportedly were drilled through fractured granite bedrock covered by a disintegrated granite soil. Sewage from the lodge was discharged to three 1500-gallon concrete septic tanks. All 3 wells were located within 92–115 feet of a septic tank or outhouse. Assessment of the sewage system indicated that new connections increased the flow of wastewater to a system not designed or constructed to effectively treat the increased volume.

Interviews of food handlers revealed that food safety training was limited and managerial controls were poor. Several food handlers also reported gastrointestinal illness during the epidemic period. No stool specimens from food handlers were available for pathogen testing.

Discussion. This report describes a multistrain, waterborne outbreak of gastroenteritis associated with NLV that was caused by contaminated well water. The outbreak continued over the course of at least 10 weeks, and the epidemic curve was consistent with a continual exposure beginning with the first reports of illness in January 2001. Because NLV has a low infectious dose and may be easily transmitted from person to person, it is likely that a number of the ill persons had secondary cases that occurred after ⩾1 guest in a party fell ill. The presence of multiple strains suggests that the water supply may have been recontaminated by incoming guests. Molecular data show that at least 2 other individuals who visited the lodge were infected with different strains of NLV that could have contaminated the water supply, causing a cycle of illness and recontamination at the lodge.

Epidemiological analysis failed to identify an association between any food items at lodge A and illness. However, the fact that several food handlers continued to work while ill and possibly contaminated prepared foods may also have contributed to the continued rate of gastroenteritis during the epidemic period. It was initially suspected that lodge A was the source of the outbreak, primarily because of the presence of fecal coliform organisms in well-water samples, and this was supported by the epidemiological investigation. Lodge B guests who visited lodge A had an increased risk of illness, compared with guests who did not visit lodge A. In addition, there was no association with illness and water consumption among lodge B guests.

Contamination of the water supply is attributed to the geological conditions of the area and to an overloaded sewage disposal system. The sandy, porous soil present at lodge A has poor adsorption qualities and permitted rapid water percolation, decreasing the soil's ability to filter and remove viruses. Any viruses reaching the fractured granite bedrock below could then be easily pulled into the groundwater well source by the well's pumping action. The lodge owner remodeled the facilities in November 2000, and the increased sewage load was not matched with a larger septic system, which caused more effluent to pass through the leach fields at a faster rate. The site of this outbreak is illustrative of the need to carefully consider local geology and not simply distance siting requirements for septic systems and wells to ensure safe drinking water.

NLVs are not currently subject to any monitoring requirements under US drinking-water regulations. Waterborne outbreaks of NLV due to sewage-contaminated drinking water have been well documented [3, 4]. Enteric viruses pose a unique challenge both in detection and in water treatment. NLV is a robust virus that may be capable of surviving chlorine concentrations that inactivate bacteria and other viruses and is poorly filtered by most soil types [6, 14]. Although this outbreak of NLV caused by contaminated well water was supported by

the presence of fecal coliforms, this is not always a reliable indicator of viral contamination. The use of epidemiological criteria is vital to determine whether a virus may be the cause. If appropriate, testing of samples from patients and water specimens for NLV should be conducted as early in the course of the outbreak as possible.

PCR methods of detecting NLV require large volumes of water to be filtered and concentrated to detect potentially low numbers of virus particles [15]. Environmental waters contain many substances that may inhibit PCR. The molecular method–based assays used in our investigation have been developed recently to remove PCR inhibitors and provide a more rapid and sensitive method for detecting human enteric viruses present in water [8–10].

Our investigation demonstrates the value of molecular methods to complement classical epidemiological and environmental investigations and further confirms the importance of NLV as a cause of waterborne illness. The development of more-sensitive methods for detecting enteric viruses in environmental water samples should encourage consideration of a standardized program to monitor for NLVs on a routine basis in drinking water, so that epidemics may be prevented.

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